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L31: Entry 11 of 41 File: USPT Oct 28, 1997

DOCUMENT-IDENTIFIER: US 5681745 A
TITLE: Biotin-binding containment systems

Brief Summary Paragraph Right (7):
Although these techniques are quite effective, each is based on the principle that the GEMs can be physically confined. However, absolute confinement is often not possible or simply impractical. More recent techniques have focused on various methods of biological containment. Potential risks associated with deliberate or unintentional release of GEMs are minimized by the use of debilitated mutant strains or non-conjugative, non-mobilized plasmids, GEMs that escape physical confinement, according to a preprogrammed genetic design, are destroyed or cannot successfully reproduce. For example, GEMs can be engineered to require an essential nutrient which is otherwise rare or non-existent in the natural environment. GEMs can also be programmed to die in the presence of compounds which are abundant outside of the laboratory setting. Additional approaches include the introduction of conditional maintenance functions into GEMs, so that their survival is dependent on the specific environments (J. L. Ramos et at., Bio/Technology 13:35-37, 1995) or growth phase (P. Klemm et at., Appl. Environ. Microbiol. 61:481-86, 1995). Thus, by inserting toxic genes transcribed from promoters responding to environmental or intracellular changes, the viability of GEMs can be controlled. Unfortunately, the overall efficiency of suicide systems typically becomes reduced over time by mutational inactivation of the lethal cassettes although some systems benefited by combining two or more different lethal genes.

Brief Summary Paragraph Right (9):
Prior studies to control the proliferation of genetically engineered microorganisms have primarily considered two basic approaches. The first approach was to use no containment system at all for the engineered microorganism. The assumption was that even if the engineered organisms survive, they would not disrupt normal ecological balances in the environment. The other approach was to develop suicide cassettes to control the survival of the genetically engineered microorganism. Variations include the induction of peptides to disrupt membrane integrity (A. K. Bej et al., Appl. Environ. Microbiol. 54:2472-77, 1988), a TOL plasmid suicide system involving induction of the gef gene to promote cell death (A. Contreras et at., Appl. Environ. Microbiol. 57:1504-8, 1991; S. Molin et at., Bio/Technology 5:1315-18, 1987) and induction of a relF gene to promote cell suicide. However, with these approaches, the levels of inducers and the formation of resistance clones due to high mutation rates, genetic instability, remain as problems.

Detailed Description Paragraph Right (7):
A biotin-binding genetic containment system has numerous advantages over more conventional approaches. First, the system is based on a different suicide function than those previously utilized, namely biotin-binding. Second, the cassette approach described can be used in a wide range of hosts including bacteria, fungi, algae, higher plants and animal cells. Third, rates of mutation appear to be significantly lower than most of those previously reported for other suicide gene and, therefore, there is better control in environmental settings. Fourth, the suicide cassette can be easily coupled to catabolic plasmids to demonstrate function.

Consequently, genetic constructs tightly couple the degradation of hazardous chemicals to cell survival. Further, the engineered organisms can be easily detected and monitored with biotin-bound conjugates due to the production of streptavidin.

Detailed Description Paragraph Right (10):
The need for new killing genes involved in regulatory circuits of programmed cell death comes from imperfect killing by one toxin operating alone. Combinations of at least two different toxic peptides can drop appearance of mutants to 10.sup.-10 and below. For example, the stv-based system shown in FIG. 1, built in the pRO1614 vector (pMB1 and pRO1600 replicon), can deliver a second suicide function in constructs already integrated within chromosome or based on R300B plasmid replicon. This could be performed in P. putida. Streptavidin fulfills all requirements of a toxin to be involved in killing systems. The rate of mutational inactivation of stv cassette is at least equal to those estimated for constructs based on a single copy gene per plasmid. Accumulation of mutants, for example with P. putida, occurs through insufficient repression of the uninduced system because of extremely low production of LacI protein.

Detailed Description Paragraph Right (12):
Streptavidin-based containment systems represent just one construct of many that can be utilized. There are a wide range of catabolic plasmids available such as NAH, OCT and TOL, and other genes such as lignin peroxidases that degrade specific classes of hazardous organic compounds, and many additional genes that are useful as suicide cassettes. These can be combined with the subject system in a matrix of ways to build a wide range of metabolic capabilities into these organisms to handle a wide range of environmental contamination problems.

Detailed Description Paragraph Right (18):
Another embodiment of the invention is directed to a nucleic acid that contains a suicide cassette which encodes a biotin-binding protein. The biotin-binding protein is preferably streptavidin and is utilized for either a suicide function or as a detection marker. The cassette may be a single nucleic acid fragment or the components of the cassette may be distributed on multiple fragments. Preferably, the nucleic acid contain convenient restriction endonuclease sites to simplify transfer of the cassette between vectors, origins of replication for maintenance, transcriptional promoters as described above to control gene expression and recognition sites for various enzymes to maximize expression when stimulated such as during transcription or translation. Useful vectors for carrying or transferring the cassette include viral vectors for eukaryotic cells (AdV, AAV, HSV), plasmids and phage vectors for bacterial cells, and shuttle vectors such as cosmids for both. Optionally, the nucleic acid cassette may also encode inhibitors of the transcriptional effector which can be utilized to reduce the chances of undesired low levels of suicide gene expression. These inhibitors may be expressed at high or low levels, depending on their effect of the host cell and the ability of the suicide gene product to quickly and efficiently overcome the inhibition upon expression. Preferably, the inhibitor is constituitively expressed at fairly low levels.

Detailed Description Paragraph Right (27):
Subcloning experiments were performed in E. coli DH5.alpha. (recA1 hsdR17 endA1 thi-1 gyrA96 relA1 supE44.o slashed.80.delta.lacZ.DELTA.M15.gamma..sup.-) (T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982). Suicide constructs were tested in the mentioned E. coli strain and Pseudomonas putida KT2440 (hsdR1 hsdM.sup.+) (F.C.H. Franklin et al., Proc. Natl. Acad. Sci. USA 18:7458-62, 1981). DNA used were plasmids pKK223-3 (Amp.sup.r) (J. Brosius et at., Proc. Natl. Acad. Sci. USA 6929-33, 1984), pLysE (Cm.sub.r) (F. W. Studier et at., Methods Enzymol. 185:60-89, 1990), pRO1614 (Amp.sup.r, Tet.sub.r) (R. H. Olsen et at., J. Bacteriol. 150:60-69, 1982), pTSA-13 (Amp.sup.r), pUC19 (Amp.sup.r) (C. Yanisch-Peron et at., Gene 33:103-19, 1985), pVLT33 (Kan.sup.r) (V. de Lorenzo et at., Gene 123:17-24, 1993), replicative form (rf) of bacteriophage mGP1-2, a derivative of pGP1-2 (S. Tabor et al., Proc. Natl. Acad. Sci. USA 82:1074-78, 1985), and synthetic oligonucleotides.

Detailed Description Paragraph Right (31):
To achieve tighter control of streptavidin synthesis, the stv gene was placed under the regulation of the bacteriophage T7 expression system (T. Sano et al., Proc. Natl. Acad. Sci. USA 87:142-46, 1990). In this case, transcription of the stv gene is controlled by the T7.o slashed.10 promoter which is recognized only by T7 RNA polymerase. Bacteriophage polymerase is provided from the T7 gene 1, placed under control of the P.sub.tac promoter, negatively regulated by the LacI repressor. To compensate for the leakiness of P.sub.tac, an inhibitor of T7 RNA polymerase, T7 lysozyme (B. A. Moffatt et al., Cell 49:221-27, 1987), was also supplied. It was expressed from the T7 gene 3.5 placed under the P. putida TOL plasmid P.sub.m, promoter. In an uninduced state in E. coli, P.sub.m behaves as a very weak constitutive promoter, allowing sufficient synthesis of T7 lysozyme to inactivate low levels of T7 RNA polymerase. The P.sub.m promoter was chosen because leaving the lysozyme gene even without promoter appeared to be sufficient to reduce leakiness of P.sub.tac. Higher levels of lysozyme in the system are designed to respond to low levels P.sub.m promoter activator which completely represses basal T7 RNA polymerase expression. Streptavidin is expressed upon inactivation of LacI with IPTG, or, if the lacI gene is fused to, for example, the P.sub.m promoter, in response to depletion of a hydrocarbon effector.

Detailed Description Paragraph Right (32):
With this design, lethal expression of the stv gene is tightly controlled by the bacteriophage T7 transcription system, that is the .o slashed.10 promoter, the RNA polymerase encoded by the T7 gene 1 fused with Escherichia coli P.sub.tac promoter, and the lysozyme, here as an inhibitor of RNA polymerase. This entire containment system can be conditioned by the E. coli lacI repressor gene fused with a promoter responding to environmental or physiological changes. A plasmid-based construct was examined in E. coli and Pseudomonas putida. Induction of stv gene expression resulted in cell-killing with efficiency up to 99.9%. Mutants escaping killing appeared at frequencies reaching 10.sup.-6 -10.sup.-7 per cell per generation. The general requirement for biotin in the living systems makes the stv cassette a candidate for containment strategies in a broad range of microorganisms.

Detailed Description Paragraph Right (36):
stv gene - T7 gene 3.5 - T7 gene I cassette: A fragment of a pTSA-13 derivative containing the T7 lysozyme and stv genes with their regulatory sequences was cut out with EcoR I and Bgl II, and blunt-ended with Klenow polymerase. This blunt-ended fragment was inserted into the blunt-ended BamH I site of the pKK derivative described above, upstream from the P.sub.tac :: T7 gene I fusion. A Sph I-Hind III fragment of the resulting plasmid pKK-slp bearing the stv cassette (FIG. 1) was finally inserted into the Sph I and Hind III sites of pRO1614, a vector for enteric bacteria and pseudomonads.

Detailed Description Paragraph Right (37):
Functionality of the stv gene-based design was analyzed in E. coli by constructing a DH50.alpha. strain carrying plasmids pKK-slp or pRO-sip. P. putida, which does not contain the lacI gene on the chromosome, was first transformed with pVLT33 (lacA) and then with pRO-sip. However, in P. putida such construct did not respond to IPTG, probably because of overproduction of LacI. Similar behavior of P. putida has been already reported. To be able to check the stv cassette in P. putida, it was inserted into a multiple cloning site of a pVLT33 derivative with lacI gene depleted from its own promoter. In this case (pVLT-lslp) transcription of lacI was very low, originating somewhere within the stv cassette. Cultures were grown overnight in LB medium supplemented with appropriate antibiotic, diluted with the same medium to A.sub.450 nm .about.0.1, and further grown to early exponential phase. As shown in FIGS. 2 and 3, the addition of IPTG at A.sub.450 .about.0.4 inhibited cell growth, both in E. coli and P. putida.

Detailed Description Paragraph Right (38):
Luria-Delbruck experiments (S. E. Luria et at., Genetics 28:491-511, 1943) were performed using 96-well

microtiter plates and carbenicillin instead of ampicillin (L. B. Jensen et at., Appl. Environ. Microbiol. 59:3713-17, 1993). The efficiency of killing of host cells by the expression of streptavidin was tested by counting viable cells before addition of IPTG, and at different times after. Bacterial samples were washed with LB medium to remove IPTG, spread on LB agar plates supplemented with appropriate antibiotic and 50 .mu.g/ml biotin, and incubated for a week. An hour after induction of streptavidin synthesis, 99.9% of E. coli and 92.3% of P. putida cells could not recover even after prolonged incubation in the presence of biotin (FIG. 3). Surviving colonies of E. coli contained a fully active suicide system, as checked on plates with and without IPTG. A progressive growth up of bacterial population after about 10 hours of exposure to IPTG resulted from the loss of the plasmid due to destruction of ampicillin by .beta.-lactamase released to the medium. In case of P. putida, all surviving clones appeared to contain mutations (probably point mutations) within the suicide system. That is why in this case cultures resumed just 3 hours after induction of the stv gene. Rates of mutational inactivation of the streptavidin-based suicide system in E. coli DH5.alpha. and P. putida KT2440 are shown in Table 1.

Detailed Description Paragraph Center (4):
Bacterial Strains, Plasmids and Culture Conditions.

CLAIMS:

4. The genetic containment system of claim 1 wherein the cassette comprises a regulatable promoter and a suicide gene.

5. The containment system of claim 4 wherein the regulatable promoter is functionally linked to said suicide gene.

8. The containment system of claim 4 wherein the regulatable promoter is selected from the group consisting of bacteriophage SP6 promoters, bacteriophage T7 promoters, bacteriophage T3 promoters, bacteriophage .gamma. p.sub.L promoters, trp promoters, lac promoters, hybrid trp-lac promoters, phoA promoters, gal promoters, metallothionein promoters, MMTV promoters and hybrids and combinations thereof.

9. The containment system of claim 4 wherein the regulatable promoter is an inducible promoter selected from the group consisting of phage inducible promoters, nutrient inducible promoters, temperature inducible promoter, radiation inducible promoters, metal inducible promoters, hormone inducible promoters, steroid inducible promoters and hybrids and combinations thereof.

10. The containment system of claim 4 wherein the regulatable promoter is regulated by a transcriptional effector.

11. The containment system of claim 10 wherein the transcriptional effector is a transcriptional repressor or a transcriptional activator.

13. The containment system of claim 10 wherein the regulatable promoter is a bacteriophage T7 promoter and the transcriptional effector is a T7 RNA polymerase.

15. The containment system of claim 10 wherein the regulatable promoter is functionally linked to a genetic element that is regulated by said transcriptional effector.

16. The containment system of claim 10 wherein the regulatable promoter is induced by one or more physiological conditions.

17. The containment system of claim 16 wherein the physiological conditions are selected from the group consisting of changes in pH, temperature, radiation, osmotic pressure, saline gradients, cell surface binding and the concentration of one or more extrinsic or intrinsic agents.

18. The containment system of claim 17 wherein the extrinsic agent is selected from the group consisting of amino acids and amino acid analogs, saccharides and polysaccharides, nucleic acids, transcriptional activators and repressors, cytokines, toxins, petroleum-based compounds, metal containing compounds, salts, ions, enzyme substrate analogs and combinations thereof.

27. The nucleic acid of claim 26 wherein the genetic vector is a plasmid, viral vector, cosmid, phage vector or combination thereof.

28. The nucleic acid of claim 26 wherein the streptavidin gene is functionally linked to a regulatable promoter.

29. The nucleic acid of claim 28 wherein the regulatable promoter is selected from the group consisting of bacteriophage SP6 promoters, bacteriophage T7 promoters, bacteriophage T3 promoters, bacteriophage .gamma. p.sub.L promoters, trp promoters, lac promoters, hybrid trp-lac promoters, phoA promoters, gal promoters, metallothionein promoters, MMTV promoters and combinations thereof.

30. The nucleic acid of claim 28 wherein the regulatable promoter is an inducible promoter selected from the group consisting of phage inducible promoters, nutrient inducible promoters, temperature inducible promoter, metal inducible promoters, hormone inducible promoters, steroid inducible promoters and combinations thereof.

31. The nucleic acid of claim 26 wherein the physiological condition is selected from the group consisting of a changes in pH, temperature, radiation, osmotic pressure, saline gradients and concentration of an extrinsic or intrinsic agent.

32. The nucleic acid of claim 31 wherein the extrinsic or intrinsic agent is selected from the group consisting of amino acids and amino acid analogs, saccharides and polysaccharides, nucleic acids, transcriptional activators and repressors, cytokines, toxins, petroleum-based compounds, metal containing compounds, salts, ions, enzyme substrate analogs and combinations thereof.

41. A genetic containment system comprising a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, a suicide control gene encoding a heterologous polymerase functionally linked to a repressible promoter regulated by a repressor, and a polymerase control gene functionally linked to a constitutive promoter.

43. The genetic containment system of claim 41 wherein the inducible promoter is a bacteriophage .gamma., SP6, T3 or T7 promoter.

45. The genetic containment system of claim 41 wherein the repressor is a lactose, galactose or tryptophan repressor protein.

46. The genetic containment system of claim 41 wherein the repressible promoter is P.sub.tac, P.sub.lac, P.sub.trp, P.sub.gal, P.sub.phoA or a hybrid thereof.

48. The genetic containment system of claim 41 wherein the constitutive promoter is P.sub.m.

49. The genetic containment system of claim 41 further comprising a gene encoding the repressor functionally linked to another inducible promoter.

50. The genetic containment system of claim 49 wherein the another inducible promoter that is activated or repressed in response to a change of an environmental condition.

52. A genetic containment system comprising a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, a suicide control gene encoding a polymerase functionally linked to a repressible promoter, a polymerase control gene functionally linked to a constitutive promoter, and a gene that encodes said repressor functionally linked to another inducible promoter that responds to an environmental condition.

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Search Results - Record(s) 1 through 8 of 8 returned.

☐ 1. Document ID: US 6316609 B1

L11: Entry 1 of 8 File: USPT Nov 13, 2001

DOCUMENT-IDENTIFIER: US 6316609 B1
TITLE: Nucleotide sequence of Escherichia coli pathogenicity islands

Detailed Description Paragraph Right (113):
Allelic knock-outs are constructed using different pir-dependent suicide vectors (Swihart, K. A. and R. A. Welch, Infect. Immun. 58:1853-1869 (1990)). In addition, two different animal model systems can be employed for assessment of pathogenic determinants. The initial identification of E. coli hemolysin as a virulence factor came from the construction of isogenic E. coli strains that were tested in a rat model of intra-abdominal sepsis (Welch, R. A. et al., Nature (London) 294:665-667 (1981)). The ascending UTI (Urinary Tract Infection) mouse model was also successfully performed with allelic knock-outs of the hptmA hemolysin of Proteus mirabilis (Swihart, K. A. and R. A. Welch, Infect. Immun. 58:1853-1869 (1990)).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6024961 A

L11: Entry 2 of 8 File: USPT Feb 15, 2000

DOCUMENT-IDENTIFIER: US 6024961 A
TITLE: Recombinant avirulent immunogenic S typhi having rpos positive phenotype

Detailed Description Paragraph Right (87):
The pir-dependent R6K replicon has been used by numerous investigators and is one of the most reliable suicide vectors available for allele replacement. Replication of the R6K plasmid requires the pir gene product. A pir-dependent plasmid will not replicate in a pir.sup.- host bacterium, and so the presence of a defined

deletion mutation on a pir-dependent plasmid will allow for the selection of rare events in which the plasmid has integrated into the host chromosome within a homologous region flanking the deletion constructed on the plasmid. This event will confer some selectable phenotype upon the strain into which the plasmid has integrated, because even though the plasmid cannot replicate, the integration event provides a mechanism of stable maintenance of the elements on the plasmid. Antibiotic-resistance elements are generally used to select for the presence of the integrated plasmid, and can be selected from genes which encode resistance to ampicillin, kanamycin, chloramphenicol, gentamicin, spectinomycin and tetracycline, and others well known in the art. The host strain which contains a defined deletion along with an integrated suicide vector is characterized as a merodiploid, since it contains two different alleles of the same gene. Generally, the deletion constructed on the vector will represent a gene deletion and the integrated product on the chromosome will have the structure characterized by the presence of a wild-type allele flanking one end of the integrated vector, and the defined deletion mutant allele at the other end of the vector. Other constructions are well known in the art.

Detailed Description Paragraph Right (102):
The .DELTA.phoPQ23 deletion was obtained by digesting pMEG-068 with EcoRV and TthIIII1 removing the 1103 bp EcoRV-TthIII1 fragment encoding the C terminal end of PhoP and the His region of PhoQ, responsible for phosphorylation of PhoP (FIG. 6). The linearized plasmid was then treated with T4 DNA polymerase and religated to produce pMEG-210 (FIG. 6). The BamHI-XbaI fragment of pMEG-210 containing the .DELTA.phoPQ23 deletion was then inserted in the pir-dependent suicide vector pMEG-149 to produce pMEG-213 (FIG. 6). Since pMEG-213 is a mobilizable suicide vector encoding for the selectable marker for ampicillin resistance and the counter-selectable marker, levanosucrase, resulting in sensitivity to sucrose, the plasmid can be conjugated into any strain desired selecting for ampicillin resistance followed by counter-selection for the replacement of the wild-type phoPQ genes with the mutant phoPQ23 in the presence of sucrose. The host strain responsible for the delivery of pMEG-213 was obtained by transforming pMEG-213 into the Pir.sup.+ Asd.sup.- delivery host MGN-617 to produce MGN-758.

Detailed Description Paragraph Right (136):
The wild-type rpoS gene can be introduced into the chromosome of MGN-1256 by allelic exchange using the suicide properties of the R6K-based plasmid pMEG-149. Plasmid pMEG-149 is a mobilizable suicide vector which carries a .lambda.pir-dependent R6K replicon and thus requires a host with the pir gene present in trans to allow replication. In addition, pMEG-149 encodes the selectable marker for Ap.sup.r and the counterselectable marker, levanosucrase. Since pMEG-149 derivatives cannot replicate in strains lacking the pir gene, selection of Ap.sup.r transconjugants demands the integration of the plasmid into the chromosome, an event which usually takes place through homology in the inserted fragment.

☐ 3. Document ID: US 5843426 A

L11: Entry 3 of 8 File: USPT Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843426 A

TITLE: Salmonella vaccines

Detailed Description Paragraph Table (9):
TABLE 15

Plasmids, strains and relevent properties S. typhimurium strains Relevent genotypes/information MSI.sup.a Source.sup.b

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ATCC14028 Wild type 3.90 ATCC CS019 phoN2 zxx::6251Tn10d-Cm (31) CS585 CS019,pagD::TnphoA 0.002 (4) CS586 CS585:phoP105::Tn10d-Tet (4) JSG205 ATCC14028, msgA::MudJ 0.01 This work JSG225 JSG205,phoP105::Tn10d-Tet This work CS811 CS019, envE::TnphoA This work CS812 CS811,phoP105::Tn10d-Tet This work CS100 ATCC14028, phoP105::Tn10d-Tet 0.01 derivitive of TT13208 JSG232 JSG205,envF::pGPP2 This work JSG244 CS019,envF::pGPP2 This work JSG235 JSG234,phoP105::Tn10d-Tet This work JSG244 JSG205,phoP105::Tn10d-Tet This work CS099 ATCC14028;zxx3024::Tn10.DELTA.16.DELTA.17pol-2(Whitfield This work polA amber) Other salmonellae Ty2 Vi positive FDA Salmonella paratyphi A ATCC 9150 ATCC Salmonella paratyphi C ATCC 13428 ATCC Salmonella enteriditis Clinical isolate VRI E. coli Strains SM10.lambda.pir thi-l thr-1 leuB6 supE44 tonA211 lacY1recA::RP4-2-Tc::Mu DH5.alpha. F-.O slashed.80dlacZ.DELTA.M15.DELTA.(lacZYA-argF)U169endA1recA 1hsdR17deoRthi-1supE44.lambda.- gyrA96relA1 Other Enterobacteriaceae Yersinia enterocolitica Clinical isolate MGH bacteriology lab Vibrio cholerae Clinical isolate Peruvian epidermic Campylobacter fetus Clinical isolate MGH bacteriolog lab Citrobater freudii Clinical isolate MGH bacteriology lab Klebsiella pneumoniae Clinical isolate MGH bacteriolagy lab Shigella flexneri Clinical isolate MGH bacteriology lab Shigella sonnei Clinical isolate MGH bacteriology lab Morganella morganii Clinical isolate MGH bacteriology lab Providencia stuartii Clinical isolate MGH bacteriology lab Plasmids pWPL17 pBR322 containing a 2.8 Kb HpaI fragment from This work pCAA9 pWPL17 containing a TnphoA insertion in envF This work pGP704 pir-dependent suicide vector (34) pGPP2 pGP704 containing the cloned envF::phoA gene This work pWP061 Cosmid clone containing the pagC region (36)

.sup.a MSI (macrophage survival index) is calculated by dividing the number of surviving organisms at 24 hours postinfection by the number of cell asscociated organisms present after the 30 minute infection. .sup.b MGH, Massachusetts General Hospital, ATCC, American Type Culture Collection, FDA, Food and Drug Administration; VRI, Virus Research Institute 4 Belden et al., 1989, Infect. Immun., 57:1-7 31 Miller et al., 1989, Proc. Natl. Acad. Sci. USA, 86:5054-58 34 Miller et al., 1988, J. Bacteriol., 170:2575-83 36 Pulkkinen et al., 1991, J. Bacteriol., 173:86-93

☐ 4. Document ID: US 5747028 A

L11: Entry 4 of 8 File: USPT May 5, 1998

DOCUMENT-IDENTIFIER: US 5747028 A

TITLE: Immunizing compositions comprising Vibrio cholerae expressing heterologous antigens

Detailed Description Paragraph Right (9):
The desired fragments were then introduced into the suicide vector pCVD442 as follows. pSAB12 and pSAB19 were digested with HindIII and EcoRI and the DNA fragment containing either the irgA deletion (from pSAB12) or the irgA deletion-slt-IB-substitution (from pSAB19) were made blunt-ended by the Klenow fragment of DNA polymerase I. Following ligation to SalI linkers, the fragments were ligated into the unique SalI site of pCVD442, yielding plasmids pSAB14 and pSAB24 respectively, and propagated in the permissive strain SM10 .lambda. pir. Plasmid pCVD442 is a recently described suicide vector containing the pir-dependent R6K replicon, ampicillin resistance, and the sacB gene from Bacillus subtilis (Donnenberg et al., Infect. Immun. 59:4310-4317, 1991).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 5. Document ID: US 5733760 A

L11: Entry 5 of 8 File: USPT Mar 31, 1998

DOCUMENT-IDENTIFIER: US 5733760 A
TITLE: Salmonella vectors encoding truncated pag fusion protein, method of making, and uses thereof

Detailed Description Paragraph Right (55):
The suicide plasmids described were RP4 replicons which require the pir protein for replication [Kolter, R., et al., Cell 15:1199-1208 (1978)]. When RP4 replicons, such as JM703.1 and pGP704, are mobilized into a strain which lacks pir, they cannot replicate and homologous recombination events can be recognized by selection of the plasmid encoded ampicillin resistance [Nakayama, K., et al., Bio/Tech. 6:693-697 (1988)]. First, pGP704 was digested with the restriction endonucleases BgIII and Xba1, end-filled using the large fragment of DNA polymerase 1, and ligated using T4 ligase to form pWPL11 (See, FIG. 1). This removed restriction endonuclease sites unique to pGP704 that could be used in the construction of synthetic DNA with restriction endonuclease sites useful for cloning heterologous DNA. An approximately three kilobase SphI/EcoRI restriction endonuclease generated DNA fragment of Salmonella DNA from pWPL10 [Miller, V. L., et al., Salmonellae. Infect. Immun. 60:3763-3770 (1992)] containing approximately 1.5 kilobases 5' and 0.8 kilobases 3' to the pagC protein coding region was inserted into the SphI and EcoRI restriction sites of pWPL11 to construct pWPL12. DNA with multiple restriction endonuclease sites was constructed by hybridization of complementary synthetic oligonucleotides. This synthetic oligomer was ligated to pWPL12 which had been digested with the restriction endonucleases ClaI and BgIII and transformed into SM10.lambda.pir.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 6. Document ID: US 5695983 A

L11: Entry 6 of 8 File: USPT Dec 9, 1997

DOCUMENT-IDENTIFIER: US 5695983 A
TITLE: Salmonella vaccines

Detailed Description Paragraph Table (9):
TABLE 15

Plasmids, strains and relevent properties Relevent genotypes/information MSI.sup.a Source.sup.b

S. typhimurium strains ATCC14028 Wild type 3.90 ATCC CS019 phoN2 zxx::6251Tn10d-Cm (31) CS585 CS019, pagD::TnphoA 0.002 (4) CS586 CS585; phoP105::Tn10d-Tet (4) JSG205 ATCC14028, msgA::MudJ 0.01 This work JSG225 JSG205, phoP105::Tn10d-Tet This work CS811 CS019, envE::TnphoA This work CS812 CS811, phoP105::Tn10d-Tet This work CS100 ATCC14028, phoP105::Tn10d-Tet 0.01 derivitive of TT13208 JSG232 JSG205, envF::pGPP2 This work JSG234 CS019, envF::pGPP2 This work JSG235 JSG234, phoP105::Tn10d-Tet This work JSG244 JSG205, phoP105::Tn10d-Tet This work CS099 ATCC14028; zxx3024::Tn10.DELTA.16.DELTA.17pol-2 This work (Whitfield polA amber) Other salmonellae Ty2 Vi positive FDA Salmonella paratyphi A ATCC 9150 ATCC Salmonella paratyphi C ATCC 13428 ATCC Salmonella enteriditis Clinical isolate VRI E. coli Strains SM10.lambda.pir thi-1 thr-1 leuB6 supE44 tonA21 lacY1recA::RP4-2-Tc::Mu DH5.alpha. F.sup.- .O slashed. 80dlacZ.DELTA.M15 .DELTA.(lacZYA-argF)U16 9endA1recA1hsdR17deoRthi-1supE44.lambda..sup.- gyrA96relA1 Other Enterobacteriaceae Yersinia enterocolitica Clinical isolate MGH bacteriology lab Vibrio cholerae Clinical isolate Peruvian epidemic Campylobacter fetus Clinical isolate MGH bacteriology lab Citrobater freundii Clinical isolate MGH bacteriology lab Klebsiella pneumoniae Clinical isolate MGH bacteriology lab Shigella flexneri Clinical isolate MGH bacteriology lab Shigella sonnei Clinical isolate MGH bacteriology lab Morganella morganii Clinical isolate MGH bacteriology lab Providencia stuartii Clinical isolate MGH bacteriology lab Plasmids pWPL17 pBR322 containing a 2.8 Kb HpaI fragment from This work pCAA9 pWPL17 containing a TnphoA insertion in envF This work pGP704 pir-dependent suicide vector (34) pGPP2 pGP704 containing the cloned envF::phoA gene This work pWP061 Cosmid clone containing the pagC region (36)

.sup.a MSI (macrophage survival index) is calculated by dividing the number of surviving organisms at 24 hours postinfection by the number of cell associated organisms present after the 30 minute infection. .sup.b MGH, Massachusetts General Hospital, ATCC, American Type Culture Collection, FDA, Food and Drug Administration; VRI, Virus Research Institute .sup.4 Belden et al., 1989, Infect. Immun., 57:1-7 .sup.31 Miller et al., 1989, Proc. Natl. Acad. Sci. USA, 86:5054-58 .sup.34 Miller et al., 1988, J. Bacteriol., 170:2575-83 .sup.36 Pulkkinen et al., 1991, J. Bacteriol., 173:86-93

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 7. Document ID: US 5686580 A

L11: Entry 7 of 8 File: USPT Nov 11, 1997

DOCUMENT-IDENTIFIER: US 5686580 A
TITLE: Antibodies having binding specificity to ShET2, an enterotoxin of Shigella flexneri 2A

Detailed Description Paragraph Right (106):
A deletion of 900 nucleotides in the virG gene (Lett et al, J. Bacteriol., 172:352-359 (1989)), which corresponds to a deletion of amino acids 341-640 of the 120 kDa VirG protein, was obtained by following steps analogous to that used for preparing the .DELTA.aroA mutation. The specific engineered site for this deletion in the 120 kDa protein represents a highly hydrophobic, poorly antigenic portion of the molecule according to the Jameson/Wolf antigenic index (IBI Pustell Sequence Analysis Programs). More specifically, the 5' end of the virG gene was amplified with the upstream primer (GGGGAATTCCAAATTCACAAATTTTTTTGT) (SEQ ID NO:9) so as to introduce an EcoRI site, and with the downstream primer (TCCATGCCATTCATGGAGTATTAATGAATT) (SEQ ID NO:10). The 3' end of the virG gene was amplified with the upstream primer (CTCCATGAATGGCATGGAAAGGCGGAATA) (SEQ ID NO:11), and the downstream primer (CGGGTCGACTCAGAAGGTATATTTCACACCCAA) (SEQ ID NO:12) so as to introduce a SalI site. Amplification and fusion of the virG 5' and 3' segments were performed using the same PCR cycles described above. The resulting new .DELTA.virG gene was cloned into the EcoRI and SAlI sites of the pir-based suicide vector pKTN701 (Hone et al, Vaccine, 9:810-816 (1991)), giving rise to pSh.DELTA.virG, which was electroporated into E. coli strain SY327 (Miller et al, J. Bacteriol., 170:2575-2583 (1983)). The plasmid was then electroporated into strain Sm10.lambda.pir (Miller et al, J. Bacteriol., 170:2575-2583 (1983)). Sm10.lambda.pir(pSh.DELTA.virG) was used to conjugate the deletion cassette into the .DELTA.aroA strain, CVD1201.1.

☐ 8. Document ID: US 5589380 A

L11: Entry 8 of 8 File: USPT Dec 31, 1996

DOCUMENT-IDENTIFIER: US 5589380 A
TITLE: Isolated DNA molecule encoding SHET1 of Shigella flexneri 2a and mutant Shigella flexneri 2a

Detailed Description Paragraph Right (116):
A deletion of 900 nucleotides in the virG gene (Lett et al, J. Bacteriol., 172:352-359 (1989)), which corresponds to a deletion of amino acids 341-640 of the 120 kDa VirG protein, was obtained by following

steps analogous to that used for preparing the .DELTA.aroA mutation. The specific engineered site for this deletion in the 120 kDa protein represents a highly hydrophobic, poorly antigenic portion of the molecule according to the Jameson/Wolf antigenic index (IBI Pustell Sequence Analysis Programs). More specifically, the 5' end of the virG gene was amplified with the upstream primer (GGGGAATTCCAAATTCACAAATTTTTTTGT) (SEQ ID NO:9) so as to introduce an EcoRI site, and with the downstream primer (TCCATGCCATTCATGGAGTATTAATGAATT) (SEQ ID NO:10). The 3' end of the virG gene was amplified with the upstream primer (CTCCATGAATGGCATGGAAAGGCGGAATA) (SEQ ID NO:11), and the downstream primer (CGGGTCGACTCAGAAGGTATATTTCACACCCAA) (SEQ ID NO:12) so as to introduce a SalI site. Amplification and fusion of the virG 5' and 3' segments were performed using the same PCR cycles described above. The resulting new .DELTA.virG gene was cloned into the EcoRI and SalI sites of the pir-based suicide vector pKTN701 (Hone et al, Vaccine, 9:810-816 (1991)), giving rise to pSh.DELTA.virG, which was electroporated into E. coli strain SY327 (Miller et al, J. Bacteriol., 170:2575-2583 (1983)). The plasmid was then electroporated into strain Sm10.lambda.pir (Miller et al, J. Bacteriol., 170:2575-2583 (1983)). Sm10.lambda.pir(pSh.DELTA.virG) was used to conjugate the deletion cassette into the .DELTA.aroA strain, CVD1201.1.

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L31: Entry 24 of 41 File: USPT Mar 23, 1993

US-PAT-NO: 5196317
DOCUMENT-IDENTIFIER: US 5196317 A

TITLE: Overexpression of proteins in recombinant host cells

DATE-ISSUED: March 23, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Heinrich; Martin	Darmstadt			DEX
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US-CL-CURRENT: 435/69.1; 435/190, 435/248, 435/252.3, 435/252.33

CLAIMS:

What is claimed is:

1. In a process for the preparation of a protein by cultivating recombinant prokaryotic host cells in a nutrient solution and by expression of the protein with a thermally-inducible promoter system and subsequent isolation of the protein, the improvement comprising carrying out the expression of the protein

(a) at a biomass of the recombinant host cells present at the beginning of induction of expression which is equivalent to 5 to 40% of the maximum achievable biomass,

(b) at a suboptimal expression temperature and

(c) in the presence of one or more added amino acids and/or a yeast extract,

whereby the thus-produced protein is biologically active.

2. A process of claim 1, wherein the recombinant host cells are E. coli.

3. A process of claim 2, wherein the expression of the protein is controlled by a lambda P.sub.L promoter and a CI857 repressor.

4. A process of claim 3, wherein the expression of the protein is carried out at a temperature of 37.degree. to 40.degree. C.

5. A process of claim 1, wherein the added amino acids comprise phenylalanine, tyrosine and tryptophan.

6. A process of claim 1, wherein the expressed protein is glucose dehydrogenase.

7. A process of claim 1, wherein the recombinant host cells are Bacillus.

8. A process of claim 2, wherein the expressed protein is glucose dehydrogenase.

9. A process of claim 8, wherein a yield of specific activity of 150 to 250 U/mg of protein is achieved.

10. A process of claim 4, wherein the expression of the protein is carried out at a temperature of 38.degree. to 39.degree. C.

11. A process of claim 1, wherein the expressed protein is derived from a prokaryote.

12. A process of claim 10, wherein the expressed protein is derived from a prokaryote.

13. A process of claim 1, wherein the suboptimal expression temperature is at least 2.degree. C. lower than the optimal expression temperature for said thermally-inducible promoter system.

14. A process of claim 12, wherein the expressed protein is glucose dehydrogenase.

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L31: Entry 9 of 41 File: USPT Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843702 A
TITLE: Gene expression system

Brief Summary Paragraph Right (15):
The invention also relates to bacterial hosts containing one or more of the expression systems or genes as defined above. The invention also provides plasmids carrying one or more of the genes defined above.

Brief Summary Paragraph Right (18):
Also provided is a method of integrating a gene into a bacterial chromosome in which a segment of phibacin DNA is inserted into a plasmid carrying the desired gene, the plasmid is introduced into a bacterial cell carrying on the chromosome at least a portion of phibacin DNA having the same or a substantially similar DNA sequence as that of the phibacin DNA segment contained on the plasmid, and recombination events between the plasmid and the phibacin DNA in the bacterial chromosome, which integrate the desired gene into the chromosome, are selected for by methods known in the art. Any segment of phibacin DNA would be suitable to drive integration.

Drawing Description Paragraph Right (2):
FIG. 1. Map of the integrating vector, pWD3, constructed as described in Materials and Methods. The Cm.sup.r gene from pBD64 was subcloned into the multiple cloning site of pUC18. This was followed by insertion of the promoterless alpha-amylase gene, isolated from pSL5 (36). The resulting plasmid contains unique restrictions sites for EcoR1, Sac1, Sma1 and BamH1 immediately 5' to the promoterless alpha-amylase gene.

Drawing Description Paragraph Right (13):
FIG. 10. The lysis negative phenotype observed upon integration of pWD35 compared to wild type lysis proficient phenotype generated upon integration of pWD38. The plasmids carry a promoterless gene for heat stable alpha amylase (HT alpha amylase) from Bacillus licheniformis (37,36).

Drawing Description Paragraph Right (14):
FIG. 11. The heat inducible expression of the gene for heat stable Bacillus licheniformis alpha amylase after heat induction of PBSX in lA420:pWD35 and comparison with a non-inducible strain and a strain harbouring a multicopy recombinant plasmid pSA33 containing the same alpha amylase gene.

Drawing Description Paragraph Right (17):
FIG. 13. Construction of the PBSX late promoter-lacZ transcriptional fusion. The 1.3 kb EcoR1-BamH1 fragment which contains the late promoter was ligated to EcoR1-BamH1 cut pDG268. The resulting plasmid was linearised using Xbal before transformation of B. subtilis. Alpha-amylase negative transformants were used for subsequent experiments.

Drawing Description Paragraph Right (18):
FIG. 14. Restriction digests of recombinant plasmids which encode the factor required for transcription from the late promoter. DNA from the PBSX early region was digested with HindIII and cloned into the HindIII site of pEB112. Plasmids were isolated from four .beta.-galactosidase producing transformants (plasmids 1-4), and transformed into E. coli. Plasmid DNA from the E. coli transformants was digested with HindIII and restriction fragments separated on an agarose gel. Lanes 1 & 2: plasmid 1; lanes 3 & 4: plasmid 2; lanes 5 & 6: plasmid 3; lane 7: plasmid 4; lane 8: 1 kb size ladder.

Drawing Description Paragraph Right (22):
Table 1: Bacterial strains, plasmids and bacteriophage (BGSC: Bacillus Genetic Stock Center).

Drawing Description Paragraph Right (23):
Table 2: Mutagenic properties of the indicated plasmids when integrated into B. subtilis L8508. Presence or absence of killing of induced cells on a lawn of B. subtilis W23. Presence or absence of cell lysis was determined by monitoring optical density.

Drawing Description Paragraph Right (24):
Table 3: Presence or absence of the major PBSX induced proteins in cells containing the indicated integrated plasmids. Data are accumulated from 10% and 13.5% SDS-polyacrylamide gels.

Drawing Description Paragraph Right (25):
Table 4: Bacterial strains and plasmids used in assessment of positive control factor.

Detailed Description Paragraph Right (1):
Bacterial strains, plasmids and phages are listed in table 1. B. subtilis IA4201 was derived by congression, using DNA from strain S0113 to transform IA420. PurA.sup.+ colonies were selected and screened for acquisition of the amy-3 mutation. The construction of the integrating vector is shown in FIG. 1. The chloramphenicol acetyl-transferase gene was removed from pBD64 on a 1.1 kb. HpaII fragment. This was treated with Klenow fragment and ligated to EcoRI and Kenlow treated pUC18. The promoterless alpha-amylase gene (36, 37) was removed from pSL5 on a BamHI-HindIII and inserted between the BamHI-HindIII sites. The EcoRI site distal to the alpha-amylase gene in the resulting plasmid was removed by a partial EcoRI digestion, treatment with Klenow fragment, followed by religation. The resulting plasmid pWD3 contains unique restriction sites for EcoRI, SacI, SmaI and BamHI immediately 5' to the promoterless alpha-amylase gene.

Detailed Description Paragraph Right (4):
B. subtilis and E. coli were routinely grown on Luria broth or agar. When appropriate, media contained chloramphenicol (3 .mu.g/ml) for selection in B. subtilis or ampicillin (50 .mu.g/ml) for selection of plasmids in E. coli. Alpha-amylase activity was detected by adding starch (0.2%) to the media and subsequently staining the plates with a solution of 0.5% I.sub.2, 1% KI.

Detailed Description Paragraph Right (7):
Small scale preparations of plasmid were obtained as described previously (3). Large scale plasmid preparations were further 35 purified by CsCl density gradient centrifugation (28).

Detailed Description Paragraph Right (10):
To label proteins in cells induced for PBSX, overnight cultures were diluted to a cell density of 6.times.10.sup.6 in SS+0.05% glucose and grown to a cell density of 3.times.10.sup.7. PBSX was induced by shifting the growth temperature to 48.degree. C. At 30 min and 40 min post-induction lml aliquots were withdrawn and incubated with continued shaking in the presence of L-[.sup.35 S] methionine. After 5 min.,

cold methionine was added (0.33 ml of 50 mg/ml). Cell pellets were stored at -20.degree. C. and processed for electrophoresis as described (31). Labelling of plasmid encoded proteins in E. coli was performed according to the method of Sancar et al. (41).

Detailed Description Paragraph Right (12):
PBSX does not package its own genome (19, 34). Therefore, in order to isolate DNA coding for phage functions, a B. subtilis 168 chromosomal bank, (constructed in lambda EMBL3), was screened with pOK411C, a plasmid which was known to contain DNA from the PBSX prophage (36). By successive cycles of screening, approximately 33 kb. of DNA from the PBSX region of the chromosome were isolated in four overlapping lambda clones. A restriction map of the cloned region is presented in FIG. 2. Hybridisation of selected fragments to chromosomal DNA digests by Southern blotting (43), indicated that no rearrangements had occurred (data not shown).

Detailed Description Paragraph Right (13):
With a view to analysing the transcriptional activity across this region, the cloned DNA was used to direct integration of a promoterless alpha-amylase gene into the chromosome. A series of fragments were subcloned into the integrating vector pWD3, using unique restriction sites immediately 5' to the promoterless alpha-amylase gene (FIG. 2). Each plasmid was transformed into B. subtilis IA4201, with selection for the acquisition of chloramphenicol resistance. In each of six cases checked, integration appeared to have occurred by a Campbell type mechanism, resulting in the plasmid sequences being flanked by direct repeats of the chromosomal DNA which directed integration (data not shown) (12, 36). Expression of the alpha-amylase gene in each of the fusion strains now provided a convenient method to assay transcriptional activity across the cloned DNA.

Detailed Description Paragraph Right (15):
In order to establish the position of the cloned DNA on the chromosome and to determine its orientation, two strains with plasmids integrated at either extremity of the cloned region, IA4201::pWD312 and IA4201::pWD316 were used as donors and recipients in PBS-1 transduction. In each case the site of integration of thechloramphenicol resistance marker was mapped with respect to neighbouring chromosomal markers (FIG. 4). The chloramphenicol resistance marker in strain IA4201::pWD312 was closely linked to phoS, a mutation which results in constitutive alkaline phosphatase expression (39). In strain IA4201::pWD316 the chloramphenicol resistance gene had integrated close to the xhi1479 allele which confers the heat-inducible phenotype (4). The data suggest the order of markers as shown in FIG. 4. Although mapping studies were not carried out with respect to other PBSX markers, correlation with the known genetic map for this region suggests that the cloned DNA spans the sites of mutations within PBSX genes coding for head (xhd) and tail (xtl, xki) proteins (4, 15, 16).

Detailed Description Paragraph Right (16):
Thermo-induction of alpha-amylase expression in the fusion strains carrying the xhi1479 allele, together with the mapping data provided strong evidence that the cloned DNA was derived from the PBSX region of the chromosome, but did not exclude the possibility that much of the DNA could lie outside the PBSX genome itself. Indeed, replication of PBSX DNA is thought to extend into flanking host DNA (2, 16, 49). In order to distinguish between these possibilities the mutagenic properties of integrating plasmids were exploited: If integration is mediated by an internal fragment of an operon, then the integration event will disrupt functions of this operon (32, 40).

Detailed Description Paragraph Right (17):
Production of PBSX particles can be detected by their bacteriocidal activity on a PBSX sensitive strain B. subtilis W23 (33,45,46). The plasmid pOK411C, isolated by O'Kane et al. (36), was shown to contain a fragment of PBSX origin by its ability when integrated to abolish this PBSX killing function. Each fragment as

shown in FIG. 2 was used to direct integration of pWD3 into B. subtilis L8508, and the resulting strains tested for production of killing activity. Of 11 strains tested, 9 showed a marked reduction in killing activity when compared to that of the parental strain (table 2). (Residual killing activity in these strains may be due to a low level of transcriptional readthrough from the plasmid sequences, or to excision of the plasmid sequences in a sub population of cells).

Detailed Description Paragraph Right (20):
Fragment 316 is derived from the extreme left hand end of the map (FIG. 2). Strain L8508::pWD316 fails to show induction of any detectable phage proteins. Integration of plasmid pWD37 prevents the synthesis of a number of phage proteins, including a major head protein and its proposed precursor X35 and P36 (29), several tail proteins, X76 and X19, and non-structural proteins P32 and P31. Proteins P36/X35, X19 and P18 are observed in strains L8508::pWD35, L8508::pWD32 and L8508::pWD312, although these strains lack X76, P32 and P31. Strains L8508::pWD38 and pWD39 produce all detectable PBSX proteins, which is consistent with the non-mutagenic nature of integration directed by these two fragments.

Detailed Description Paragraph Right (21):
Integration of plasmids pWD37, pWD32, pWD35 and pWD312 prevented production of some, but not all of the phage structural proteins, which suggested that the respective fragments might be derived from a late operon of the prophage. In order to examine if these fragments contained the genes for any known phage proteins, plasmids pWD37, pWD32 and pWD35 were transformed into E. coli CSR603. Plasmid encoded proteins produced in maxi-cells were labelled and separated on 8% and 12.5% SDS-PAGs (FIG. 6). In the strain containing pWD35 proteins were observed which corresponded in size to previously identified phage proteins X58, X19 and P17. In addition this fragment encoded a protein which corresponded in size to a newly identified phage protein which we have named X59. It can be seen when phage particle proteins are separated on low percentage gels. Plasmid pWD32 encoded a protein which corresponded in size to a phage tail protein, (X22), while pWD37 gave rise to a protein which corresponded to P14, a protein of unknown function found in induced cells. Proteins X58 and X19 were produced in sufficient amounts to be subjected to limited proteolysis by the method of Cleveland (7). In each case peptides of similar size to those of the phage proteins were produced, thus confirming their identity (data not shown).

Detailed Description Paragraph Right (23):
The late operon indicated is thought to be at least 18 kb in length. The restriction map suggests that fragments within the region 8 kb to 26 kb direct mutagenic integration, indicating that each is derived from an internal part of an operon. If these fragments are contiguous, as the restriction map suggests, then these fragments must be derived from the one operon. Within this proposed operon, genes have been assigned to regions based on the assumption that in each strain transcription will proceed only to the end of the fragment which directs integration in each case, i.e. the 3' end of the operon, downstream from the integrated plasmid sequences in each case will not be transcribed. Thus, a protein absent in one integrant strain, but present in a strain containing the plasmid integrated further downstream, can be assigned to the intervening region. (e.g. Protein X19 is absent in strain L8508::pWD37, but present in L8508::pWD35 and hence the gene for this protein has been tentatively assigned to fragment 35.)

Detailed Description Paragraph Right (25):
Fragments of DNA from the PBSX region of the chromosome were cloned into a replicating plasmid pRP22, and tested for their ability to allow the strain B. subtilis IA4201, which is thermoinducible for PBSX, to grow at the non-permissive temperature. A clone containing a 1.2 kb fragment was isolated which when cloned into pUB110 was capable of complementing the xhi1479 allele. This fragment was therefore expected to encode the wild-type repressor protein.

Detailed Description Paragraph Right (39):

Tests for lysis, and for induction and expression of alpha-amylase, in B. subtilis strains, carrying various plasmids integrated in the PBSX genome were performed in the absence of antibiotics. Overnight cultures were inoculated into 200 ml of Luria Broth in a side-arm flask and grown in an orbital incubator (Gallenkamp) at 200 rpm. Optical density, as a measure of cell numbers and therefore of growth and lysis, was measured on a Klett-Summerson photoelectric colorimeter with a green filter number 54. For thermoinduction experiments cultures were split in two and one half shifted to 48.degree. C., the other half maintained at 37.degree. C. The time of induction of each culture is shown in appropriate figures (10-12). In the case of rapid thermoinduction flasks were placed in a shaking waterbath for 8 minutes at 48.degree. C., for gradual induction they were placed directly into an orbital incubator at the same temperature. Aliquots were removed at intervals prior to and post induction and centrifuged at 3,000 g for 15 minutes, the pellets were retained for chromosomal DNA preparation and alpha amylase assays were performed on the supernates.

Detailed Description Paragraph Right (40):
FIG. 10 shows growth curves for IA4201::pWD35 and IA4201::pWD38 and the effect of temperature induction at 2 hours. After heat induction the latter strain lyses, but the former does not. This shows that certain integrants, of which IA4201::35 is an example, are deficient in lysis after induction of PBSX. This is due to mutagenesis caused by integration of derivatives of the non-replicative plasmid pWD3 carrying segments of the PBSX genome, in this case pWD35. The strain carrying pWD38 is not defective in lysis. This experiment establishes that integration at certain sites in the PBSX genome blocks lysis. Table 2 is a summary of the data for various integrants.

Detailed Description Paragraph Right (41):
FIG. 11 shows that on raising the temperature of the culture to 48.degree. C. at time zero the strain IA4201::pWD35 which carries the heat inducible PBSX mutation xhi1479 begins to express the HT amylase gene from pWD35, which is integrated in the PBSX genome. In a control strain not carrying the xhi1479 allele HT alpha amylase expression was not expressed at a detectable level. In a second control strain LA2, which carries the HT amylase gene on a plasmid pSA33 (37) where it is expressed under the control of its own promoter, the level of expression of the HT gene is less and is not heat inducible.

Detailed Description Paragraph Right (45):
Plasmid pJG14 (Devine et al, J. Bacteriol. 1989., 171, 1166) was used as a .sup.32 p radiolabelled probe. This carries the CAT gene which is also part of all the derivatives of pWD3, and a segment of the chromosomal .beta.-glucanase gene. It can therefore be used to measure the copy number of the pWD35 sequences integrated in the PBSX genome relative to the copy number of chromosomal sequences not linked to PBSX. Chromosomal DNA preparations from IA4201::pWD35 were made from cells harvested at different times before and after heat induction. Heat induction was carried out under regimes as described above, rapid induction and gradual induction. The DNA was digested with EcoR1 and electrophoresed on a 0.8% agarose gel. Southern blot and hybridisation on Biodyne membranes was performed as advised by the manufacturers. Autoradiography was performed on probed filters. Copy number was estimated by comparing the intensity of the signal produced by DNA within the PBSX region to DNA from another part of the chromosome (.beta.-glucanase). Signal intensity was measured on a DESAGA Chromatogram Densitometer CD50.

Detailed Description Paragraph Right (50):
Transcription from the late promoter was monitored by using a transciptional fusion to the .beta.-galactosidase structural gene. The PBSX late promoter P.sub.L, was removed from pWD38 on a 1.3 kb EcoR1-BamH1 fragment and cloned between EcoR1 and BamH1 sites of DG268, immediately 5' to a promoterless lacZ gene. The resulting plasmid was linearised using Xbal and used to transform B. subtilis strains RB1081 and 1A420. Integration of the plasmid into the chromosome by a double recombination event results in disruption of the .beta.-amylase coding sequence by the integrated P.sub.L -lacZ fusion (see FIG. 13). This resulted in strains RB1081[P.sub.L -lacZ] and 1A420[P.sub.L -lacz]. In each case the late

promoter-lacZ fusion is integrated at the amylase locus which is quite distinct from the PBSX prophage.

Detailed Description Paragraph Right (52):
In order to isolate the gene encoding this factor, DNA from a plasmid which contains a 15 kb insert spanning the PBSX early region (pHV1435h), was digested with HindIII. Fragments were ligated to the E.coli--B. subtilis shuttle vector, pEB112(cut with HindIII), and transformed into B. subtilis RB1081[P.sub.L -lacZ]. Kanamycin resistant transformants were screened for production of .beta.-galactosidase. Four such transformants were isolated, each of which was noted to grow poorly, and when restreaked on fresh plates, segregated white colonies. Each of the recombinant plasmids contains a 1.5 kb HindIII fragment. One of these plasmids was named pWH15. It is not known whether the observed instability is due to the cloned insert; the vector, pEB112, has previously been reported to be unstable in B. subtilis.

Detailed Description Paragraph Right (54):
A molecular genetic analysis of the PBSX prophage has been carried out by examining the effects of integrating plasmid sequences at different sites within the metA-metC region of the B. subtilis 168 chromosome. Insertional mutagenesis has allowed the identification of early and late regions of the prophage. The relative positions of these regions within the cloned region is consistent with proposed genetic map of this region which suggests that mutations within a regulatory region (xin and xhi), are located to the metA proximal side of mutations within genes affecting particle proteins (xhd, xtl, xki) (4, 15, 49).

Detailed Description Paragraph Right (57):
By integrating the promoterless alpha-amylase gene at the PBSX locus the potential for using PBSX functions as the basis of a thermoinducible expression vehicle has been demonstrated. Plasmid based thermoinducible expression systems have been described for B. subtilis which exploit the controlling elements of early region of acteriophage phi105 (10, 38). The system presented here demonstrates that a foreign gene can be integrated on the B. subtilis chromosome under the control of an inducible prophage promotor. Furthermore the foreign gene is located within a structure reported to the capable of undergoing stable gene amplification (24). Indeed stable maintenance of both single and amplified copies of the alpha-amylase gene have been observed when integrated at this locus.

Detailed Description Paragraph Right (81):
3. Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nuc. Acids Res. 7: 1513-1523.

Detailed Description Paragraph Right (87):
9. Contente, S., and D. Dubnau. 1979. Characterisation of plasmid transformation in Bacillus subtilis: kinetic properties and the effect of DNA conformation. Mol. Gen. Genet. 167: 251-258.

Detailed Description Paragraph Right (95):
17. Gryczan, T., S. Contente, and D. Dubnau. 1980. Molecular cloning of heterologous chromosomal DNA by recombination between a plasmid vector and a homologous resident plasmid in Bacillus subtilis. Mol. Gen. Genet. 177: 459-467.

Detailed Description Paragraph Right (114):
36. O'Kane, C., M. A. Stephens, and D. J. McConnell. 1986. Integrable alpha-amylase plasmid for generating transcriptional fusions in Bacillus subtilis. J. Bacteriol. 168: 973-981.

Detailed Description Paragraph Right (118):
40. Piggot, P. J., C. A. Curtis, and H. de Lencastre. 1984. Use of integrational plasmids to demonstrate the polycistronic nature of a transcriptional unit (spoIIA) required for sporulation of Bacillus subtilis. J. Gen.

Microbiol. 130: 2123-2136.

Detailed Description Paragraph Right (119):
41. Sancar, A., A. M. Hach and W. D. Rupp. 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. 137: 692-693.

Detailed Description Paragraph Left (1):
Bacterial Strains, Plasmids and Phases

Detailed Description Paragraph Left (13):
Production of PBSX Proteins in Strains Containing Integrated Plasmids

Detailed Description Paragraph Left (27):
Bacterial strains and plasmids are listed in table 4.

Detailed Description Paragraph Table (1):
TABLE 1 _____ Plasmid, strain or bacteriophage Genotype Source or reference _____ Plasmid: pBD64 Cm.sup.R (16) pUC18 Ap.sup.R (44) pSL5 Ap.sup.R, -amy structural gene (31) pOK411C Cm.sup.R, -amy structural gene (31) pWD3 Cm.sup.R Ap.sup.R, -amy structural This study. gene E. coli: DH5.alpha. F.sup.- end.sup.A1 hsd.sup.R17 (r.sub.K .sup.-, m.sub.K .sup.+) sup.sup.E44 thi.sup.-1 lambda.sup.- rec.sup.A1 Bethseda Research gyr.sup.A96 rel.sup.A1 o80dlacZ.DELTA.M15 Laboratories. Nm539 sup.sup.F hsd.sup.R (P2cox3) (12), Promega Biotec. CSR603 F.sup.- thr.sup.-1 leu.sup.B6 pro.sup.A2 phr.sup.-1 rec.sup.A1 arg.sup.E3 thi.sup.-1 uvr.sup.A6 ara.sup.-14 lac.sup.Y1 gal.sup.K2 sup.sup.E44 mtl.sup.-1 rps.sup.L31 tsx.sup.-33 xyl.sup.5 lambda.sup.- R. L. Rodriquez. B. subtilis 168: SO113 trp.sup.C2 amy.sup.-3 (32) IA420 ilv.sup.A1 met.sup.B5 pur.sup.A16 xhi.sup.1479 xki.sup.1479 (4), BGSC. IA4201 ilv.sup.A1 met.sup.B5 xhi.sup.1479 xki.sup.1479 amy.sup.-3 This study. L8508 xhi.sup.1479 lyt.sup.-2 D. Karamata. SL345 pho.sup.S5 leu.sup.A8 rif.sup.-2 spollE64 R. Buxton. IA78 met.sup.C3 pyr.sup.A xtl.sup.-1 BGSC. IA158 met.sup.A BGSC. B. subtilis W23: SB623 thr(PBSZ) BGSC. Bacteriophage: Lambda EMBL3 (12), Promega Biotec. PBS-1 BGSC. _____

Detailed Description Paragraph Table (2):
TABLE 2 _____ Integrated Killing Activity Cell Lysis upon Plasmid on B. subtilis W23 PBSX Induction _____ None + + 316 - - 38 + + 37 - - 35 - - 314 - - 313 - - 32 - - 31 - - 311 - - 312 - - 39 + +
_____

Detailed Description Paragraph Table (3):
TABLE 3 _____ PROTEIN INTEGRATED PLASMID (m. wt.) 316 38 37 35 32 312 39 _____ X76(Tail) - + - - - - + P70 - + + + + + + P36 - + - + + + + X35 - + - + + + + (Head) P32 - + - - - - + P31 - + - - - - + X19(Tail) - + - + + + + P18 - + - + + + + p14 - + + + + + + _____

CLAIMS:

4. The expression system as claimed in claim 1 further comprising DNA sequences which encode a repressor, a promoter, and at least one operator, isolated from a phibacin selected from the group consisting of PBSW, PBSX, PBSY, and PBSZ of Bacillus subtilis.

6. A method of integrating a DNA sequence of interest into a Bacillus chromosome, in which a segment of DNA from an expression system as claimed in claim 1 is inserted into a plasmid carrying the DNA sequence to

be integrated, the plasmid is introduced into a bacterial cell carrying on the chromosome at least a portion of phibacin DNA having the same or substantially the same DNA sequence as that of the phibacin DNA in the plasmid, whereby recombination between the phibacin DNA in the plasmid and in the chromosome is accomplished, thereby integrating the DNA sequence of interest into the chromosome, and integrants are selected.

9. The expression system as claimed in claim 4 wherein the phibacin carries a temperature sensitive repressor allele.

10. The expression system of claim 9 wherein the temperature sensitive allele is the xhi1479 mutation.

12. The expression system as claimed in claim 4 wherein the DNA sequence which encodes the repressor is the gene designated orf1 of phibacin PBSX in FIG. 8(SEQ ID NO 1), or mutants thereof having retained the ability to encode protein having repressor function.

13. The expression system as claimed in claim 4 wherein the promoter has the DNA sequence shown in FIG. 15, between the -10 and -35 consensus sequences, or a mutant thereof having retained the ability to act as a promoter.

15. The expression system as claimed in claim 4 comprising a DNA sequence encoding a temperature sensitive repressor so that product expression is heat-inducible.

17. The expression system as claimed in claim 15 wherein said DNA sequence is the xhi1479 allele of the gene designated orf1of PBSX in FIG. 9(SEQ ID NOS 3 and 5), or mutants thereof having retained the ability to encode a protein having heat-inducible repressor function.

19. A DNA sequence isolated from a phibacin selected from the group consisting of PBSW, PBSX, PBSY and PBSZ of Bacillus subtilis, which encodes a repressor, or a mutant thereof having retained the ability to encode a protein having repressor function.

21. The DNA sequence claimed in claim 19 contained in the phibacin in Bacillus subtilis SO113 deposited with the National Collection of Industrial Bacteria, under the accession no. NCIMB 40205, or a mutant of the sequence which encodes a protein having a repressor function and/or a mutant of the sequence encoding a positive control factor.

22. The DNA sequence of claim 20 comprising the gene designated orf1 in FIG. 8(SEQ ID NO 1), or mutants thereof having retained the ability to encode a protein having repressor function.

23. The DNA sequence of claim 22 wherein the repressor encoded is temperature sensitive.

24. The DNA Sequence of claim 23 which is the temperature sensitive xhi1479 allele in FIG. 9(SEQ ID NOS 3 and 5) of the gene designated orf1 of PBSX or mutants thereof which encode a protein having repressor function.

26. The DNA sequence claimed in claim 25 contained in the phibacin in Bacillus subtilis 1A4201 deposited with the national Collection of Industrial Bacteria, under the accession no. NCIMB 40206, respectively, or a mutant of the sequence which encodes a protein having a repressor function and/or a mutant of the sequence encoding a positive control factor.

28. A plasmid carrying a DNA sequence according to claim 21.

29. A plasmid carrying a DNA sequence according to claim 26.

WEST Search History

DATE: Friday, April 05, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	pr near5 c2	34	L1
L2	c1857 or c-1857	111	L2
L3	L2 and temperature	106	L3
L4	L3 and plasmid	105	L4
L5	L4 and asd	0	L5
L6	L4 and (pola or pol-a)	0	L6
L7	L4 and p22	1	L7
L8	L4 and lambda	102	L8
L9	L8 and (pmeg$ or p-meg$)	0	L9
L10	L8 and pir	7	L10
L11	pir near10 suicide	8	L11
L12	L11 and l2	0	L12
L13	c2 same l2	0	L13
L14	c2	68552	L14
L15	L14 same pir	13	L15
L16	L15 not l11	13	L16
L17	pir-dependent or pirdependent	5	L17
L18	L17 not l11	0	L18
L19	mgn417 or mgn-417	0	L19
L20	pmeg	44	L20
L21	pmeg096 orpmeg-096	0	L21
L22	elvs	121	L22
L23	elvs!	24	L23

L23	elvs!		24	L23
L24	L23 and plasmid		7	L24

END OF SEARCH HISTORY

WEST

Generate Collection Print

Suicide vectors

L27: Entry 12 of 29 File: USPT Mar 21, 2000

DOCUMENT-IDENTIFIER: US 6039941 A
TITLE: Live vaccine for the treatment of tumor diseases

CLAIMS:

1. A therapeutic agent comprising live tumor cells which have been genetically modified to comprise:

a) an operon comprising a cytokine gene and sequences necessary for its expression, and

b) an operon comprising an immunostimulatory membrane protein gene and sequences necessary for its expression, wherein the genetically modified tumor cells are autologous or allogeneic tumor cells capable of proliferation. wherein the cytokine gene codes for a substance which induces the differentiation, proliferation and activation of immune cells, and wherein the immunostimulatory membrane protein gene codes for a membrane protein that stimulates T cells.

2. The therapeutic agent of claim 1 further comprising an operon with a suicide gene and sequences necessary for its expression.

3. The therapeutic agent of claim 2, wherein the suicide gene codes for an enzyme which can convert a non-toxic compound into a toxic product.

4. The therapeutic agent of claim 3, wherein the suicide gene is selected from the group consisting of the thymidine kinase gene of herpes simplex virus and cytosine deaminase gene.

7. The therapeutic agent of claim 1, wherein one or more of the sequences necessary for the expression of the cytokine gene and the immunostimulatory membrane protein gene causes constitutive expression.

11. A method of making a therapeutic agent comprising live tumor cells which have been genetically modified to comprise: a) an operon comprising a cytokine gene and sequences necessary for its expression, and b) an operon comprising an immunostimulatory membrane protein gene and sequences necessary for its expression, said method comprising:

a) obtaining autologous or allogenic tumor cells; and

b) introducing into said tumor cells at least two additional genes wherein said additional genes are a cytokine gene and an immunostimulatory membrane protein gene, wherein the cytokine gene codes for a substance which induces the differentiation, proliferation and activation of immune cells, and wherein the

immunostimulatory membrane protein gene codes for a membrane protein that stimulates T cells.

13. The method of claim 11 wherein a suicide gene is also introduced into said tumor cells.

14. A therapeutic agent comprising live tumor cells which have been genetically modified to comprise:

a) an operon comprising a cytokine gene and sequences necessary for its expression, and

b) an operon comprising an immunostimulatory membrane protein gene and sequences necessary for its expression, wherein the genetically modified tumor cells are autologous or allogeneic tumor cells capable of proliferation, wherein the cytokine is IL-4 or IL-7, and wherein the immunostimulatory membrane protein gene is the gene for T cell costimulatory molecule B7.

15. The therapeutic agent of claim 14, further comprising an operon with a suicide gene and sequences necessary for its expression.

WEST

Generate Collection Print

L27: Entry 15 of 29 File: USPT Aug 3, 1999

DOCUMENT-IDENTIFIER: US 5932210 A
TITLE: Recombinant adenoviral vector and methods of use

CLAIMS:

1. A composition comprising a recombinant adenovirus expression vector and a pharmaceutically acceptable carrier, the vector comprising:

(a) an insert of exogenous DNA comprising a gene encoding a foreign protein; and

(b) adenovirus DNA in which all of the coding sequences of E1a, E1b, and protein IX, and at least part of E3 have been deleted.

8. The composition of claim 1, wherein the foreign protein is a suicide protein or functional equivalent thereof.

9. The composition of claim 1, wherein the gene encoding the foreign protein is expressed under control of a cytomegalovirus (CMV) promoter.

10. The composition of claim 1, wherein the gene encoding the foreign protein is expressed under control of an adenovirus promoter.

WEST

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L25: Entry 14 of 15 File: USPT Nov 1, 1988

DOCUMENT-IDENTIFIER: US 4782022 A
TITLE: Nitrogen fixation regulator genes

CLAIMS:

3. A recombinant DNA plasmid as recited in claim 1 wherein said promoter comprises the nucleotide sequence

5'-A--
A--G--C--T--T--A--A--A--C--C--T--G--C--C--T--C--G--C--G--C--T--C--A --C--G--C--G--A--G--
T--C--T--T--G--C--C--C--G--G--C--C--A--A--A--T--G--C--T--A--C--G--A--G--T-
-T--G--A--A--T--G--A--A--A--
C--T--G--G--G--C--A--A--G--T--G--G--A--A--G--T--C--A--C--T--G--C--C--G--A-
-T--G--G--C--T--G--C--A--T--
G--G--A--G--T--G--C--G--G--C--A--C--A--T--G--C--A--G--A--G--T--G--T--T--G-
-T--G--C--G--A--G--G--C--A--
A--A--C--G--G--T--G--A--C--G--T--C--G--A--G--T--G--G--A--G--C--T--A--T--C-
-C--A--C--G--A--G--G--T--G-- G--C--T--T--C--G--G--T--G--T--C--C--T--C--T--T--C--A--A--G--T--T--C--G--
G--A--T--G--A--G--C--C--A--C--
T--C--T--A--A--G--G--T--C--G--A--T--T--C--A--C--A--A--G--C--T--A--G--A--T-
-C--G--G--C--G--T--T--C--A--
A--T--A--G--G--G--G--G--A--C--G--A--A--G--T--G--C--C--A--G--G--G--A--T--C-
-C--T--T--A--C--A--A--G--A--
A--C--C--A--A--C--T--T--A--C--C--T--T--C--C--G--T--A--A--C--T--T--T--A--T- -C--G--C--T--C--T--C--C--G--
A--C--T--G--T--C--A--A--T--A--C--G--C--A--T--A--C--C--T--C--C--T--A--A--T-
-A--T--T--A--A--G--C--G--G--
G--C--G--A--G--A--A--A--A--T--G--A--C--T--A--A--G--G--T--G--C--T--C--C--C-
-A--T--C--G--C--A--A--C--T-- C--G--T--T--C--A--G--G--G--G--A--G--T--T-- A--G--T--G--C--C--C--T--G--T--C
--T--G--T--A--C--C--T--T--C--
A--C--A--A--A--G--A--G--A--C--A--T--G--C--G--C--A--A--A--C--A--G--G--A--C-
-A--A--G--C--G--C--T--C--C--
G--C--C--G--A--A--A--T--T--T--A--C--A--G--C--A--T--A--T--C--A--A--A--G--G- -C--T--C--T--G--3'

or a functionally equivalent sequence hybridizable thereto under stringent conditions.

4. A recombinant DNA plasmid as recited in claim 1 wherein said foreign structural gene is a bacterial toxin gene of Bacillus thuringiensis.

6. A recombinant DNA as recited in claim 5 wherein said vector functions are derived from a suicide vector.

7. A recombinant DNA as recited in claim 6 wherein said suicide vector comprises pSUP1011 and said recombinant DNA comprises a transposon Tn5.

8. A recombinant DNA plasmid as recited in claim 5 wherein said promoter is a promoter of a kanamycin resistance gene.

9. A recombinant DNA plasmid as recited in claim 5 wherein said promoter of said kanamycin resistance gene comprises the nucleotide sequence

---

5'-A--C--A--G--C--A--A--G--C--G--A--A--C--C--G--G--A--A--T--T--G--C--C--A- -G--C--T--G--G--G--G--G--C--G--
C--C--C--T--C--T--G--G--T--A--A--G--G--T--T--G--G--G--A--A--G--C--C--C--T-
-G--C--A--A--A--G--T--A--A--
A--C--T--G--G--A--T--G--G--C--T--T--T--C--T--T--G--C--C--G--C--C--A--A--G-
-G--A--T--C--T--G--A--T--G-- G--C--G--C--A--G--G--G--G--A--T--C--A--A--3'

---

or a functionally equivalent sequence hybridizable thereto under stringent conditions.

10. A recombinant DNA plasmid as recited in claim 5 wherein said fixD gene comprises the nucleotide sequence

---

5'-A--T--G--G--C--C--C--C--C--A--C--T--C--G--T--C--T--T--G--A--G--A--C--C-
-A--C--G--C--T--T--A--A--C--A--
A--T--T--T--C--G--T--G--A--A--T--A--C--C--C--T--C--T--C--T--T--T--G--A--T- -T--C--T--G--C--G--C--A--T--
G--C--G--C--C--G--C--G--G--C--G--G--A--C--T--C--G--A--G--A--T--T--C--C--G- -G--C--G--T--C--G--G--A--A--
G--G--A--G--A--G--A--C--A--A--A--G--A--T--A--A--C--A--G--C--G--G--C--T--A-
-C--C--C--G--C--A--A--C--A-- G--C--G--G--G--T--C--T--C--C--T--T--C--T--G--C--C--G--C--T--G--A--T--T--A-
-T--A--C--T--G--T--A--C--C--A-- A--A--G--G--C--C--G--C--A--A--T--A--G--A--C--C--A--A--G--
T--C--A--T--G--A--C--T--G--C--C--G--G--G--C--
G--G--C--T--G--G--T--C--G--T--A--C--C--A--G--A--C--G--T--T--T--G--C--A--A- -C--T--C--T--G--A--G--C--T--
G--T--T--C--A--A--G--G--A--T--C--A--G--A--T--A--A--A--A--T--G--G--C--G--C-
-G--G--A--A--T--T--G--G--T-- C--C--G--A--C--T--G--C--C--T--T--C--A--T--C--G--C--T--G--C--G--G--C--G--G-
-T--G--G--A--G--G--T--C--G--
A--T--C--A--C--G--A--A--A--C--G--G--G--C--G--G--A--A--T--G--C--T--G--T--G--T--G-
-G--T--T--C--G--A--G--T--G--
C--G--C--C--G--A--A--G--A--G--T--C--C--G--A--T--T--A--T--G--A--T--T--A--T-
-G--A--G--G--A--G--G--A--G-- G--T--A--C--A--C--T--T--T-- C--T--T--T--C--T--A--T--G--G--C--C--G--C--C--A
--A--T--C--T--T--G--C--G--G--
G--G--A--G--G--G--C--C--A--T--T--C--G--G--C--T--T--C--A--T--C--G--C--A--C- -A--A--T--C--A--G--C--A--G--
G--C--G--T--C--A--G--C--G--G--A--C--A--T--T--T--G--C--C--G--A--A--G--A--G-
-C--A--G--C--A--A--G--A--A--
C--A--A--C--A--G--A--A--T--T--C--A--C--G--T--G--A--T--G--A--G--C--A--G--A-
-G--C--C--A--G--A--G--T--T-- C--C--G--C--C--C--C--G--C--C--A--G--C--G--G--C--T--G--C--T--C--A--A--G--A--A-
-T--G--A--C--G--G--G--A--T--
C--A--T--C--G--G--G--G--A--A--A--G--T--A--C--C--G--C--C--C--T--C--A--T--G- -A--C--G--G--C--G--G--T--A--

G--A--T--A--C--C--G--C--C--A--A--A--G--T--C--A--T--G--G--C--A--G--A--G--A-
-C--C--A--A--T--T--C--A--A--
T--C--G--T--T--C--T--C--C--T--T--A--G--G--G--G--A--G--A--A--A--C--A--G--G-
-A--A--C--T--G--G--C--A--A--
G--G--A--A--T--G--C--T--T--T--C--C--G--A--A--G--C--T--A--A--T--C--C--A--C- -C--A--G--C--A--T--T--C--G--
A--C--T--C--G--G--C--A--A--A--A--A--A--A--G--C--C--C--T--T--C--A--T--C--A-
-A--G--T--T--C--A--A--T--T-- G--C--C--C--C--G--C--G--C--T--G--T--C--T--G--A--G--A--G--C--C--T--T--C--T-
-C--G--A--A--T--C--A--G--A-- G--C--T--G--T--T--T--G--G--A--C--A--T--G--A--G--A--A--A--G--G--T--G--C--
G--T--T--C--A--C--C--G--G--G--
G--C--T--A--T--T--G--C--T--C--A--A--C--G--A--G--T--A--G--G--C--C--G--T--T-
-T--C--G--A--A--T--C--G--G--
C--G--A--A--T--G--G--C--G--G--A--A--C--G--T--T--G--C--T--G--C--T--C--G--A-
-T--G--A--A--A--T--C--G--G-- C--G--A--G--A--T--T--C--C--C--C--C--G--G--C--G--T--T--C--C--A--A--G--C--A-
-A--A--A--C--T--G--C--T--A--
C--G--C--G--T--A--A--T--A--C--A--G--G--A--A--G--G--T--G--A--A--T--T--T--G-
-A--G--C--G--A--G--T--C--G--
G--C--G--G--C--A--C--A--A--A--G--A--C--G--C--T--G--A--A--A--G--T--C--G--A- -C--G--T--C--C--G--G--C--T--
C--A--T--A--T--T--C--G--C--C--A--C--A--A-- A--T--A--A--G--G--A--T--C--T--C
--G--A--A--A--T--G--G--C--G--
G--T--C--C--A--G--A--A--T--G--G--G--G--A--G--T--T--C--A--G--G--G--A--A--G-
-A--C--C--T--T--T--A--C--T-- A--C--C--G--C--A--T--C--A--G--C--G--G--G--G--T--G--C--C--C--C--T--C--A--T-
-T--T--T--G--C--C--G--C--C-- C--C--T--T--A--G--G--C--A--C--C--G--C--G--A--C--G--G--T--G--A--C--A--T--T-
-C--C--G--C--T--C--C--T--T-- G--C--A--A--G--A--G--C--A--T--T--C--C--T--T--C--A--G--C--G--G--T--T--C--A-
-A--C--G--A--A--G--A--G--A-- A--C--G--G--T--C--G--T--G--A--T--C--T--C--C--A--T--T--T--C--G--C--G--C--C-
-G--T--C--T--G--C--G--C--T-- T--G--A--C-- C--A--C--T--T--G--T--C--G--A--A--G--T--G--C--A--A--G--T--T--C
--C--C--T--G--G--A--A--A--C--
G--T--T--C--G--C--G--A--G--C--T--G--G--A--A--A--A--C--T--G--T--G--T--G--C-
-G--G--A--G--G--A--C--T--G--
C--A--A--C--T--C--T--C--G--C--C--A--G--G--T--C--A--A--A--G--A--C--G--A--T- -C--A--C--T--T--T--C--C--T--C--
A--G--A--T--T--T--C--G--C--C--T--G--C--C--A--A--A--C--G--G--A--C--C--A--G- -T--G--T--T--T--T--T--C--T--
T--C--T--C--G--C--C--T--C--T--G--G--A--A--A--G--G--C--G--T--T--C--A--C--T- -G--T--T--C--G--C--A--T--G--
G--C--C--A--C--A--T--T--G--A--G--A--T--C--C--A--T--G--C--G--C--C--C--G--C- -G--G--G--T--
A--C--A--A--C-- A--C--C--G--T--T--G--C--T--C--G--G--A--G--C--G--C--C--A--G--C--C--A--A--T-
-G--A--C--G--T--T--C--C--G-- C--C--G--A--A--A--G--A--G--C--C--C--C--G--G--A--T--C--C--G--C--A--G--G--A--G-
-T--G--G--C--A--T--C--C--A-- A--T--C--T--G--A--T--C--G--A--G--C--G--C--G--A--C--C--G--C--T--T--G--A--T-
-C--A--G--T--G--C--G--C--T--
G--G--A--G--G--A--G--G--C--C--C--G--G--T--T--G--G--A--A--T--C--A--G--G--C--A-
-A--A--G--G--C--A--G--C--T-- C--G--C--A--T--C--C--T--C--G--A--A--A--A--A--A--C--G--C--C--C--C--G--G--C-
-A--G--G--T--C--G--G--G--C-- T--A--T--G--C--T--C--T--A--C--G--T--C--G--G--C--A--T--G--
G--T--G--T--G--G--A--C--G--T--G--A--G--A--A--
A--G--C--T--C--T--A--A--G--C--T--G--C--C--G--G--T--G--A--3'

---

or a functionally equivalent sequence hybridizable thereto under stringent conditions.

11. A method for activating expression of nitrogen fixation genes by placing said nitrogen fixation genes under control of a constitutively expressed activating gene product comprising the steps of

(a) combining a promoter of a constitutive gene in such a position as to control a fixD gene of a Rhizobium species, wherein said activating gene codes for a product normally capable of activating R. meliloti nifHDK and

fixABC promoters, thereby producing a constitutive expression of the activating gene product,

(b) transforming a strain of Escherichia coli with a constitutive gene transfer system comprising a suicide vector and a transposon wherein the constitutively expressed activating gene is inserted within the transposon,

(c) transferring said constitutive gene transfer system to a strain of gram-negative bacteria having said nitrogen fixation genes, and

(d) selecting a recombinant strain of said gram-negative bacteria wherein said constitutively expressed activating gene is contained, replicated, and expressed in said gram-negative bacteria,

thereby activating fixation of dinitrogen by activating expression of said nitrogen fixation genes.

21. A bacterial strain as recited in claim 20 wherein said vector functions are derived from a suicide vector.

22. A bacterial strain as recited in claim 21 wherein said suicide vector comprises pSUP1011 and said vector comprises a transposon Tn5.

WEST

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L31: Entry 17 of 41 File: USPT Jul 1, 1997

US-PAT-NO: 5643790
DOCUMENT-IDENTIFIER: US 5643790 A

TITLE: Plasmid vector and a method for regulation of gene expression using the same

DATE-ISSUED: July 1, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Morinaga; Yasushi	Yokohama			JPX
Tsuchiya; Makoto	Kawasaki			JPX

US-CL-CURRENT: 435/252.3; 435/252.32, 435/320.1

CLAIMS:

What is claimed as new and desired to be secured by Letters Patent of the United States is:

1. A recombinant plasmid vector which replicates and expresses in a coryneform bacterial cell, comprising:

a) a base sequence obtained from E. coli which functions as a promoter in a coryneform bacterium;

b) a base sequence obtained from E. coli which functions as an operator downstream from said base sequence a), said operator being selected from the group consisting of a base sequence of a lac operator, a trp operator, a .lambda. operator and an operator of a phosphatase operon;

c) a base sequence obtained from E. coli which functions as a site for ribosome binding in a coryneform bacterium; and

d) a base sequence functioning as a translation initiation codon and a heterologous gene which is operably linked to said base sequence a), said vector further comprising a gene obtained from E. coli coding for a repressor protein which binds to said sequence b) functioning as an operator, and wherein the binding of said repressor protein is artificially regulated in a growth medium.

2. A bacterium containing therein the plasmid vector of claim 1, comprising:

a) a base sequence functioning as a promoter in a coryneform bacterium;

b) a base sequence functioning as an operator downstream from said base sequence a), said operator being selected from the group consisting of a base sequence of a lac operator of a trp operator, a .lambda. operator and an operator of a phosphatase operon;

c) a base sequence functioning as a site for ribosome binding in a coryneform bacterium; and

d) a base sequence functioning as a translation initiation codon and a heterologous gene, which is operably linked to said base sequence a), said vector further comprising a gene coding for a repressor protein which binds to said sequence b) functioning as an operator, and wherein the binding of said repressor protein is artificially regulated in a growth medium.

3. The vector of claim 1, wherein said translation initiation codon is ATG or GTG.

4. The vector of claim 1, wherein said heterologous gene is obtained from Streptomyces, Saccharomyces, Escherichia, or Bacillus.

5. The vector of claim 1, wherein said gene obtained from E. coli coding for a repressor protein is obtained from lac repressor, trp repressor, temperature sensitive .lambda. repressor, or the repressor of phosphatase operon.

6. A recombinant plasmid vector which replicates and expresses in a coryneform bacterial cell, comprising:

a) a base sequence obtained from E. coli which functions as a promoter in a coryneform bacterium;

b) a base sequence obtained from E. coli which functions as an operator downstream from said base sequence a), said operator being selected from the group consisting of a base sequence of a lac operator, a trp operator, a .lambda. operator and an operator of a phosphatase operon;

c) a base sequence obtained from E. coli which functions as a site for ribosome binding in a coryneform bacterium; and

d) a base sequence functioning as a translation initiation codon and a heterologous gene, which is operably linked to said base sequence a).

7. A bacterium containing therein the plasmid vector of claim 6, which replicates and expresses in a coryneform bacterial cell, comprising:

a) a base sequence obtained from E. coli which functions as a promoter in a coryneform bacterium;

b) a base sequence obtained from E. coli which functions as an operator downstream from said base sequence a), said operator being selected from the group consisting of a base sequence of a lac operator, a trp operator, a .lambda. operator and an operator of a phosphatase operon;

c) a base sequence obtained from E. coli which functions as a site for ribosome binding in a coryneform bacterium; and

d) a base sequence functioning as a translation initiation codon and a heterologous gene, which is operably linked to said base sequence a),

wherein said bacterium contains a gene obtained from E. coli coding for a repressor protein which binds to said sequence b) functioning as an operator, and wherein the binding of said repressor protein is artificially regulated in a growth medium.

8. The vector of claim 7, wherein said translation initiation codon is ATG or GTG.

9. The vector of claim 7, wherein said heterologous gene is obtained from Streptomyces, Saccharomyces, Escherichia, or Bacillus.

WEST Search History

DATE: Friday, April 05, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	pr near5 c2	34	L1
L2	c1857 or c-1857	111	L2
L3	L2 and temperature	106	L3
L4	L3 and plasmid	105	L4
L5	L4 and asd	0	L5
L6	L4 and (pola or pol-a)	0	L6
L7	L4 and p22	1	L7
L8	L4 and lambda	102	L8
L9	L8 and (pmeg$ or p-meg$)	0	L9
L10	L8 and pir	7	L10
L11	pir near10 suicide	8	L11
L12	L11 and l2	0	L12
L13	c2 same l2	0	L13
L14	c2	68552	L14
L15	L14 same pir	13	L15
L16	L15 not l11	13	L16
L17	pir-dependent or pirdependent	5	L17
L18	L17 not l11	0	L18
L19	mgn417 or mgn-417	0	L19
L20	pmeg	44	L20
L21	pmeg096 orpmeg-096	0	L21
L22	elvs	121	L22

L23	elvs!	24	L23
L24	L23 and plasmid	7	L24
L25	suicide.clm. and plasmid.clm.	15	L25
L26	suicide.clm. and (express$ or system$).clm.	41	L26
L27	L26 not l25	29	L27
L28	promoter.clm. and repress$.clm.	215	L28
L29	L28 and plasmid	208	L29
L30	L29 and l2	4	L30
L31	L29 and temperature.clm.	41	L31
L32	L28 and (high and low and temperature).clm.	4	L32

END OF SEARCH HISTORY